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EUROPEAN ATOMIC ENERGY COMMUNITY - EURATOM

AN EXPERIMENTAL ANALYSIS OF THE SPECIFICITY OF ACTIVELY ACQUIRED TOLERANCE IN MICE

by

G. DORIA

1963

Directorate General for Research and Training
Department of Biology

Paper presented at the "International Symposium on Homotransplantation"
Padova - Italy, 6-8 May 1963

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SUMMARY

Tolerance to CBA skin was induced in C3H mice by neonatal injection of CBA spleen cells. When 2 months old, the C3H recipients were grafted with CBA skin. These skin grafts showed no signs of rejection during the observation time of 3 months, whereas CBA skins grafted onto C3H mice non injected at birth showed complete necrosis in 11.7 days. Normal C3H and C3H mice tolerant to CBA skin were injected with rat RBC and sacrified 12 days later for serum titration of anti-rat RBC agglutinins. The agglutinin titer was the same in both groups. This indicates that the unresponsiveness of C3H mice to CBA skin was specific, for the tolerant mice were able to respond with normal vigor to antigens (rat RBC) unrelated to C3H and CBA. Whether this response was due to the host immune system or to the CBA spleen cells which may have colonized the C3H newborns was subsequently investigated. Spleen cells from tolerant C3H mice sensitized to rat RBC were injected into 2 groups of lethally irradiated recipients : C3H mice preimmunized against CBA and CBA mice preimmunized against C3H. Both groups were given rat RBC immediately after the spleen cell transfer from the tolerant mice and sacrified a week later for serum titration of anti-rat RBC agglutinins. These agglutinins, due to the secondary response of the transferred spleen cells, could be detected only in the group of C3H recipients preimmunized against CBA. This shows that anti-rat RBC agglutinins in tolerant mice were produced by the immune system of the C3H host. The theoretical implications of this finding will be discussed.

1 — THE PROBLEM

Billingham and Brent (1) were able to induce tolerance of skin homografts in mice by neonatal injection of spleen cells from adult mice of the skin donor strain. They also showed that tolerance is a strain specific unresponsiveness, for mice of one strain, say C3H, which had been made tolerant of skin grafts from mice of a second strain, say CBA, were able to reject with normal vigor skin homografts from mice of a second donor strain, say A.

Mice made tolerant of homografts by neonatal injection of spleen cells can be considered cell chimeras. The detection of donor type antigens in the hemopoietic tissues of tolerant mice several months after the neonatal injection of homologous spleen cells suggests that the injected donor cells can indeed grow and colonize the hemopoietic tissues of the young mouse (2), as is known to occur in other species spontaneously (3) or experimentally (4). Definitive evidence for the existence of cell chimerism was presented by Trentin and Session (5), who were able to identify donor cells by use of a chromosomal marker. On one hand the finding of antibody-forming cells in the spleen of adult mice (6), and, on the other hand the observation of runt disease (interpreted as being due to a graft-anti-host immune reaction) in tolerant mice of some donor-recipient strain combinations (7), strongly indicate that immunologically competent donor cells can colonize the injected newborns. Thus, the possibility that tolerant mice have an immune system partially or completely of donor type raises the question of whether the immune response against unrelated antigens, which defines the specificity of tolerance, is due to the host or the transplanted cells. The solution of this problem should make it possible to determine whether the specificity of tolerance is a property of the host or donor immune system, and therefore to establish whether the definition of tolerance so far referred to the whole animal (4) could also be applied to the host immune system.

2 — THE EXPERIMENT

Induction of tolerance. C3H newborn mice of both sexes were injected intravenously with 1×10^7 nucleated spleen cells from CBA adult female mice. Two months after the neonatal injection, 22 C3H mice were grafted with CBA skin from adult donors of the same sex as the recipients. The skin grafts showed no macroscopic signs of rejection during the observation time of 3 months. In contrast, CBA skins grafted onto 12 age-control C3H mice non injected at birth showed complete necrosis in 11.7 ± 0.4 days.

The specificity of tolerance was tested as follows. When 5 months old, 11 tolerant and 10 normal C3H mice were injected intraperitoneally with 1 ml of 1 % rat RBC. Twelve days later, blood was collected individually for serum titration of anti-ratRBC agglutinins. The mean $\log_2$ titer was 8.0 ± 0.3 for tolerant mice and 7.6 ± 0.2 for normal mice.

These results indicate that the unresponsiviness of C3H mice to CBA skin was specific, for the tolerant mice were able to respond with normal vigor to antigens (rat RBC) unrelated to the C3H and CBA strains.

None of the tolerant mice showed any sign of runt disease at any time before and after the injection of rat RBC.

Identification of the immune system of tolerant mice. Whether antibody-forming cells were of host or donor origin was previously investigated in mouse radiation chimeras of homologous constitutions. The immune system was shown to be of donor type (8). The same experimental design was used in the present study to identify the type (host or donor) of antibody-forming cells responsible for the production of anti-rat RBC agglutinins in tolerant C3H mice. Technical details have been presented elsewhere (9).

Normal C3H and CBA adult mice were immunized by intraperitoneal injection of 1×10^7 nucleated spleen cells from adult CBA or C3H mice, respectively, and 10 days later were given a total-body X ray dose of 700 r. Within 2 hours after irradiation, the immunized mice of each type were divided in four groups. A control group received intravenously 1 ml of Tyrode's solution. The others received intravenously one of the following inocula in 1 ml volume of Tyrode's solution : 24×10^6 nucleated spleen cells from C3H, CBA or tolerant C3H mice. All donor mice had been injected intraperitoneally with 1 ml of 1 % rat RBC 12 days earlier, and each cell suspension was prepared from a pool of 5-6 spleens. Immediately after the intravenous injection, all groups were given intraperitoneally 1 ml of 1 % rat RBC. Six days later, the recipients were decapitated, and blood was collected individually for serum titration of anti-rat RBC agglutinins. The results are presented in the following table.

ANTI-RAT RBC AGGLUTININS IN THE SERUM OF RECIPIENT MICE

Irradiated recipients given rat RBC	Spleen cell donors sensitized against rat RBC			
	none	C3H	CBA	Tolerant C3H
C3H anti CBA	0 (8)	10.5 ± 0.2 (15)	0 (7)	8.9 ± 0.1 (15)
CBA anti C3H	0 (9)	0 (12)	10.4 ± 0.2 (14)	0 (12)

mean $\log_2$ titer ± standard error (number of recipients)

The table shows that the anti-rat RBC agglutinins detected in the irradiated recipients were produced only by the secondary response of the transferred spleen cells. Spleen cells from C3H or CBA donors did not produce agglutinins when transferred into irradiated recipients specifically immunized to the spleen cell donor. Spleen cells from tolerant C3H mice produced agglutinins when transferred into irradiated C3H mice immunized to CBA tissues, but did not yield any agglutinin titer when transferred into irradiated CBA mice immunized to C3H tissues. This finding demonstrates that the secondary response of spleen cells from the tolerant mice was due only to the C3H cells, that is to the host cells.

Detection of donor antigens in the spleen of tolerant mice. If tolerant mice are cell chimeras, the injection of their tissues (containing donor type antigens) into normal mice of the host strain should elicit transplantation immunity against tissues of the donor strain (10).

Spleen cells from tolerant mice were injected into normal C3H mice, which were then tested for transplantation immunity against CBA antigens by a method fully described in a previous paper (9). The results indicated that the spleen of tolerant mice contained CBA antigens. The transplantation immunity anti-CBA elicited in the C3H mice by injection of 1×10^7 spleen cells from tolerant mice was comparable to that induced by 1×10^4 spleen cells from normal CBA mice.

3 — DISCUSSION

In the experiments presented, a normal agglutinin response against rat RBC was found in C3H mice made tolerant of CBA skin grafts by neonatal injection of CBA spleen cells. Spleen cells from tolerant mice sensitized to rat RBC were able to give a secondary agglutinin response to rat RBC when transferred into proper recipients and challenged again with the test antigen. Only cells of host type were found responsible for the agglutinin production by the transferred spleen cells. Since the identification test was based on a secondary agglutinin response, it follows that also the primary response to rat RBC in the tolerant animals should have been produced by cells of the host type. The non-detectability of immunologic activity of donor type cells raises the question of the sensitivity of the method used. Preliminary controls showed that, in order to detect a secondary agglutinin response against rat RBC in the serum of irradiated CBA mice preimmunized to C3H tissues and given the test antigen, at least 5×10^5 spleen cells needed be transferred from CBA mice sensitized against rat RBC. However, it should be pointed out that the sensitivity of the method for detecting the immunologic activity of CBA cells grown in a tolerant C3H host may be different, and therefore cannot be estimated from this type of controls. The present finding that the agglutinin response to rat RBC in tolerant mice was produced by an immune system of host type implies that immunologically competent donor cells either were absent at the time of the rat RBC challenge or, if present in sufficient number to be detected, were incapable of responding to this antigen. Both possibilities have been discussed previously (9). The fact that no immune activity of donor type could be detected might seem in disagreement with a study by Michie et al. (11). These authors analized the chimerism of the immune system of tolerant mice, making use of Simonsen's G.V.H. assay (12), whereby immunologic competence of cells is evaluated by measuring their capability to induce spleen enlargement when injected into susceptible recipients. They reported that cells from mice supposed to be *specifically* tolerant could induce spleen enlargement when transplanted into recipient mice carrying antigenic components unrelated to the host and donor strains used in the induction of tolerance. In some experiments, the transplanted cells responsible for the spleen enlargement were found to be of host type, while, in other experiments, of both host and donor types. This result would suggest that host and donor type cells potentially capable of a primary immune response were both present in some of the tolerant mice. However, since their mice had not been tested for the specificity of tolerance, it is difficult to predict which type of immune system would actually have responded in the tolerant animals, had they been challenged with antigens unrelated to the host and donor strains.

Since in the present study the host immune system was found to be the only one responsible for the primary agglutinin production elicited in tolerant mice by rat RBC, such an immune system can be considered in a tolerant state (specific unresponsiveness), for it was capable, while tolerating a homologous skin graft, to respond with normal vigor to antigens unrelated to the host and donor strains.

The finding of donor type antigens in the spleen of tolerant mice need not conflict with the non-detectability of immunologically competent cells of donor type. This may simply reflect a difference in the sensitivity of the techniques used. It is also possible that only those cells, which are not immunologically competent, have survived in the tolerant animal. Antibody-forming cells, indeed, my have selectively disappeared from the cell population injected at birth, by a mechanism of self-destruction during their reaction against the host. This mechanism, shown by Boyse (13) to occur in transplantation experiments, is still compatible with the fact that no runt disease was noted in the present study. The fact that C3H and CBA mice have the same allele at the H-2 locus would suggest indeed a very weak graft-anti-host reaction, which may not produce gross evidence of runt disease. On the other hand, the detection of donor type antigens may just indicate that cell products of donor origin were present in the spleen of tolerant mice. This possibility is suggested by the observation that foreign proteins can last for a long time in animals made specifically unresponsive (14).

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